REMARKS

Claims 1, 38, 41, and 56 are pending in the present application. Claims 38 and 41 have been amended. Claim 56 has been newly added. No new matter has been introduced by the present amendments to the claims.

Rejection under 35 U.S.C. § 101

Claims 1, 38 and 41 have been rejected under 35 U.S.C. § 101 for lack of utility. The Examiner has stated that Applicants previously submitted arguments were not deemed persuasive. The Examiner alleges that the Applicants' data submitted in previously filed Exhibit A was not disclosed in the instant specification as the time the application was filed and, as such, cannot be used to support an assertion of utility. (*See* page 3 of the Office Action). Applicants traverse.

Applicants are permitted to submit after filing evidence showing utility as long as there is an appropriate nexus to the subject matter of the specification. MPEP 2107 states:

> (3) If the applicant has not asserted any specific and substantial utility...[t]he 35 U.S.C. 101 and 112 rejections shift the burden of coming forward with evidence to the applicant to:
>
> (i) Explicitly identify a specific and substantial utility for the claimed invention; and
>
> (ii) Provide *evidence* that one of ordinary skill in the art would have recognized that the identified specific and substantial utility was well-established at the time of filing. The *examiner should review any subsequently submitted evidence of utility using the criteria outlined above*. The examiner should also ensure that there is an adequate nexus between the evidence and the properties of the now claimed subject matter as disclosed in the application as filed. That is, the applicant has the burden to establish a probative relation between the submitted evidence and the originally disclosed properties of the claimed invention. (Emphasis added).

Exhibit A has a nexus with the subject matter of the specification. The present application describes differential expression of NOV3b in human glioma, astrocytoma, renal, breast and ovarian carcinomas, and melanoma (*See* instant specification at page 91, lines 15-18). Exhibit A provides additional support of this differential expression. Therefore, Applicants assert that the data in Exhibit A has a probative relationship with the as filed specification, and, the data in Exhibit A can (and should) be used to provide evidence that a specific and substantial asserted utility or a well established utility of the claimed protein existed at the time of filing.

The utility of SEQ ID NO:8, demonstrated by the specification as filed and Exhibit A shows that SEQ ID NO:8 is useful in differentiating between pathological and normal tissues and cells, specifically renal tissue and cells.

Previously filed Exhibit A demonstrates differential expression of NOV3b in normal renal tissue and renal cancer tissue. (*See* previously filed Exhibit A and page 5, lines 5-15 of the Response to the September 2, 2002 Office Action, Paper #16). This data supports the statement on page 91, lines 15-18 of the as filed specification, that NOV3b is differentially expressed in renal cancer cells versus normal renal cells.

Applicants further submit herewith a Declaration under 37 C.F.R. 1.132 by Dr. Sasha Guo (the "Guo Declaration") asserting that the NOV3b nucleic acid encoding SEQ ID NO:8 is more highly expressed in brain cancer (astrocytoma and glioma), renal, ovarian, and melanoma cancer cell lines and in clinical specimens of ovarian, lung, kidney, liver, breast and bladder cancers as compared to surgically resected margins or normal tissue. (*See* Guo Declaration in Table AG, page 6; Table AH, pages 8-9, and Table AK, page 14 (glioma and astrocytoma versus brain), Table AG, page 7; Table AI, pages 9-10; and Table AJ, page 12; (renal cancer versus normal kidney), Table AG, page 8; Table AI, page 9; and Table AJ, page 13; (ovarian cancer versus normal ovary), and Table AG, pages 7-8; Table AI, page 9; and Table AJ, pages 12-13; (breast cancer versus normal mammary tissue)). Dr. Guo further asserts that expression of this gene is useful to differentiate these pathological cells and tissues from normal tissue and as a diagnostic marker for the presence of these cancers (*See* paragraphs 6 and 7, on page 2 of the Guo Declaration). The assertions supported by the Guo Declaration can be found in the as filed specification (*See e.g.* page 91, lines 15-18). Thus, there is a nexus between the specification and the Guo Declaration. In summary, Exhibit A and the Guo Declaration show that SEQ ID NO:8 has a specific utility that one of ordinary skill in the art will recognize for differentiating between brain, renal, ovarian and breast cancerous and normal cells and tissues.

The Examiner also alleges that the evidence presented in Exhibit A is not applicable to the utility of the peptide (SEQ ID NO:8). The Examiner alleges that since only the expression of the nucleic acid is disclosed, the data only supports utility of the nucleic acid, not the polypeptide. Applicants again traverse.

The Examiner cites Pennica *et al.* PNAS USA **95**:14717-14722 (1998) ("Pennica") as evidence that one of ordinary skill in the art would not associate mRNA transcript expression

with corresponding protein expression. The Examiner states that because Pennica shows, in one instance, that gene copy number does not correlate with overexpression of RNA, that one of ordinary skill in the art would not correlate mRNA expression with corresponding protein expression. Applicants respectfully disagree.

Applicants submit that one of ordinary skill in the art would expect that, in general, there is correlation between transcript copy number and corresponding protein expression. The standard for a specific or substantial utility is defined in MPEP 2107, which reads:

> (1) Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial. The *prima facie* showing must contain the following elements:
>
> (i) An explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is not both specific and substantial nor well-established;
>
> (ii) Support for factual findings relied upon in reaching this conclusion; and
>
> (iii) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.
>
> (Emphasis added)

First, Pennica does not disclose protein expression at all. Therefore, it does not demonstrate a relationship (or lack thereof) between protein expression and corresponding mRNA expression.

Second, the lack of correlation between the gene number and RNA expression was surprising to Pennica. The title of the subsection of the Results that the Examiner cites is, "Amplification and Aberrant Expression of WISPs in Human Colon Tumors." (Emphasis added). Thus, it was surprising to Pennica (and therefore the skilled artisan) that the mRNA transcript level was not higher, especially since the gene which encoded it was duplicated. Thus, Pennica demonstrates that the skilled artisan more likely than not would expect there is a direct correlation between transcript level and corresponding protein expression.

Applicants further submit a second 37 C.F.R. 1.132 Declaration by Dr. Seth Ettenberg (the "Ettenberg Declaration"). The Ettenberg Declaration has a probative relationship with the specification as filed in the instant Application. (*See* page 91, lines 15-19 of the specification). Therefore, it can (and should) be used to provide specific and substantial asserted utility or a well established utility of the claimed protein. The Ettenberg Declaration shows that SEQ ID NO:8

has a specific utility for differentiating between brain and renal cancerous and normal cells and tissues. Specifically, the Ettenberg Declaration shows that SEQ ID NO:8 protein was detected via FACS methods using a polyclonal antiserum raised against the protein. Specifically NOV3b was detected in certain cancer cell lines which also showed increased expression of the nucleic acid encoding SEQ ID NO:8 by RTQ-PCR analysis. Examples include glioma (U251 and SNB-19), renal (RXF393, 786-0), and lung (HOP62) cancer cell lines. Other cell lines that did not show increased expression of polynucleotide encoding SEQ ID NO:8 by RTQ-PCR also did not show expression of NOV3b protein by FACS analysis. Examples included prostate (PC3) and colon (HCT-15). (*See* paragraph 7 and Table 1 of the Ettenberg Declaration).

Applicants submit that at least one substantial and specific utility exists for the claimed invention and is readily apparent based on the teachings of the specification and is further demonstrated by the Declarations of Dr. Guo and Ettenberg. Therefore this rejection should be withdrawn.

Rejection under 35 U.S.C. § 112

Claims 1, 38 and 41 have been rejected under 35 U.S.C. § 112, first paragraph, for not providing a readily apparent use for the polypeptide of SEQ ID NO:8. Arguments made above in reference to the rejection under 35 U.S.C. § 101 apply to this 35 U.S.C. § 112 rejection as well. If the above rejection is withdrawn, this rejection will also be withdrawn.

Claims 38 and 41 have also been rejected under 35 U.S.C. § 112, first paragraph, for not being enabled. The Examiner asserts that the specification is not enabling for a pharmaceutical composition. To facilitate prosecution, Applicants have amended claims 38 and 41 to delete the word "pharmaceutical". The specification enables compositions comprising the polypeptide of SEQ ID NO:8. (*See* page 135, line 11 to page 136, line 11 of the specification). Therefore, Applicants request that this rejection be withdrawn.

CONCLUSION

On the basis of the foregoing remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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TRA 1846179v2

APPENDIX

Quantitative expressi n analysis of clones in various cells and tissues

The quantitative expression of various NOV genes was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ-PCR) performed on an Applied Biosystems (Foster City, CA) ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System.

RNA integrity of all samples was determined by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs (degradation products). Control samples to detect genomic DNA contamination included RTQ-PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

RNA samples were normalized in reference to nucleic acids encoding constitutively expressed genes (i.e., β-actin and GAPDH). Alternatively, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation, Carlsbad, CA, Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10 μg of total RNA in a volume of 20 μl or were scaled up to contain 50 μg of total RNA in a volume of 100 μl and were incubated for 60 minutes at 42°C. sscDNA samples were then normalized in reference to nucleic acids as described above.

Probes and primers were designed according to Applied Biosystems *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default reaction condition settings and the following parameters were set before selecting primers: 250 nM primer concentration; 58°-60° C primer melting temperature (Tm) range; 59° C primer optimal Tm; 2° C maximum primer difference (if probe does not have 5' G, probe Tm must be 10° C greater than primer Tm; and 75 bp to 100 bp amplicon size. The selected probes and primers were synthesized by Synthegen (Houston, TX). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: 900 nM forward and reverse primers, and 200nM probe.

Normalized RNA was spotted in individual wells of a 96 or 384-well PCR plate (Applied Biosystems, Foster City, CA). PCR cocktails included a single gene-specific probe and primers set or two multiplexed probe and primers sets. PCR reactions were done using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles: 95° C 10 min, then 40 cycles at 95° C for 15 seconds, followed by 60° C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) and plotted using a log scale, with the difference in RNA concentration between a given sample and

the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression was the reciprocal of the RNA difference multiplied by 100. CT values below 28 indicate high expression, between 28 and 32 indicate moderate expression, between 32 and 35 indicate low expression and above 35 reflect levels of expression that were too low to be measured reliably.

Normalized ssDNA was analyzed by RTQ-PCR using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification and analysis were done as described above.

Panels 1 and 1.1

Panels 1 and 1.1included 2 control wells (genomic DNA control and chemistry control) and 94 wells of cDNA samples from cultured cell lines and primary normal tissues. Cell lines were derived from carcinomas (ca) including: lung, small cell (s cell var), non small cell (non-s or non-sm); breast; melanoma; colon; prostate; glioma (glio), astrocytoma (astro) and neuroblastoma (neuro); squamous cell (squam); ovarian; liver; renal; gastric and pancreatic from the American Type Culture Collection (ATCC, Bethesda, MD). Normal tissues were obtained from individual adults or fetuses and included: adult and fetal skeletal muscle, adult and fetal heart, adult and fetal kidney, adult and fetal liver, adult and fetal lung, brain, spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. The following abbreviations are used in reporting the results: metastasis (met); pleural effusion (pl. eff or pl effusion) and * indicates established from metastasis.

Panels 2D and 2.2

Panels 2D and 2.2 included 2 control wells and 94 wells containing RNA or cDNA from human surgical specimens procured through the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI), Ardais (Lexington, MA) or Clinomics BioSciences (Frederick, MD). Tissues included human malignancies and in some cases matched adjacent normal tissue (NAT). Information regarding histopathological assessment of tumor differentiation grade as well as the clinical stage of the patient from which samples were obtained was generally available. Normal tissue RNA and cDNA samples were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics and Invitrogen (Carlsbad, CA).

Panel 3D

Panel 3D included two controls, 92 cDNA samples of cultured human cancer cell lines and 2 samples of human primary cerebellum. Cell lines (ATCC, National Cancer Institute (NCI), German tumor cell bank) were cultured as recommended and were derived from: squamous cell carcinoma of the tongue, melanoma, sarcoma, leukemia, lymphoma, and

epidermoid, bladder, pancreas, kidney, breast, prostate, ovary, uterus, cervix, stomach, colon, lung and CNS carcinomas.

A. NOV3b

Expression of gene Acc. No. 10129612.0.405 encoding SEQ ID NO: 8 was assessed using the primer-probe sets Ag2679, Ag2728, Ag2975, Ag047, and Ag47b, described in Tables AA, AB, AC, AD, AE, and AF. Results of the RTQ-PCR runs are shown in Tables AG, AH, AI, AJ, and AK. Table AA. Probe Name Ag2679

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-ttgacctcaggaacggtttac-3'	21	1294	
Probe	TET-5'-ctgctgcccaggaatacttttctccag-3'-TAMRA	26	1330	
Reverse	5'-agtatttggagggcttcttcag-3'	22	1369	

Table AB. Probe Name Ag2728

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-ggagcttggtctcatgaccta-3'	21	5181	
Probe	TET-5'-actgggctcctggccaccaagag-3'-TAMRA	23	5212	
Reverse	5'-agtcgtccatcctgtttcatc-3'	21	5236	

Table AC. Probe Name Ag2975

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-ggagcttggtctcatgaccta-3'	21	5181	
Probe	TET-5'-actgggctcctggccaccaagag-3'-TAMRA	23	5212	
Reverse	5'-agtcgtccatcctgtttcatc-3'	21	5236	

Table AD. Probe Name Ag332

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-gctgccctgacttgtgcaa-3'	19	2582	
Probe	TET-5'-tctgacccagtgtgcatctcccgtt-3'-TAMRA	25	2605	
Reverse	5'-ccggtctggcagacacact-3'	19	2639	

Table AE. Probe Name Ag047

Primers	Sequences	Length	Start Positi n	SEQ ID No
Forward	5'-ccaatgacctggccacca-3'	18	1145	
Probe	TET-5'-ccagagtccgttcagcttcaggacagc-3'-TAMRA	27	1165	
Reverse	5'-gtggcacgttgctgtttagc-3'	20	1197	

Table AF. Probe Name Ag47b

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-gaacgccggagcatacaga-3'	19	1732	
Probe	TET-5'-ccaggtactgcacaaacacggcttcat-3'-TAMRA	27	1763	
Reverse	5'-gatgccacaggcccaca-3'	17	1791	

Table AG. Panel 1

Column A - Rel. Exp.(%) Ag047, Run 87354354 Column B - Rel. Exp.(%) Ag047, Run 87354779 Column C - Rel. Exp.(%) Ag332, Run 98747043 Column D - Rel. Exp.(%) Ag47b, Run 88164379				
Tissue Name	A	B	C	D
Endothelial cells	0.0	0.0	0.0	0.0
Endothelial cells (treated)	0.0	0.0	0.0	0.0
Pancreas	0.0	0.3	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	0.0	0.0
Adrenal gland	0.0	1.3	0.0	0.0
Thyroid	0.0	0.4	0.1	0.0
Salivary gland	0.0	0.2	0.0	0.0
Pituitary gland	0.0	0.1	0.0	0.0
Brain (fetal)	0.0	15.0	11.0	21.8
Brain (whole)	95.9	67.8	4.5	32.5
Brain (amygdala)	0.0	8.8	8.7	12.2
Brain (cerebellum)	0.0	22.2	0.1	14.3
Brain (hippocampus)	0.0	24.7	8.7	15.6
Brain (substantia nigra)	3.4	3.6	2.3	3.8
Brain (thalamus)	3.5	4.7	5.0	9.1
Brain (hypothalamus)	0.0	0.7	0.0	0.0

Spinal cord	0.7	1.5	1.4	1.1
glio/astro U87-MG	0.6	2.6	1.7	3.0
glio/astro U-118-MG	0.0	0.6	1.1	0.1
astrocytoma SW1783	33.7	29.5	42.9	36.1
neuro*; met SK-N-AS	0.0	0.0	0.0	0.0
astrocytoma SF-539	31.0	36.9	68.3	48.3
astrocytoma SNB-75	33.7	32.8	23.0	50.3
glioma SNB-19	**100.0**	**100.0**	**100.0**	**100.0**
glioma U251	49.0	44.1	57.8	41.5
glioma SF-295	6.0	8.1	19.8	8.7
Heart	61.1	26.8	70.7	39.8
Skeletal muscle	0.0	0.1	0.0	0.0
Bone marrow	0.0	0.1	0.0	0.0
Thymus	18.7	18.4	2.7	17.2
Spleen	0.0	0.0	0.0	0.0
Lymph node	0.0	0.2	0.0	0.0
Colon (ascending)	0.5	0.7	0.9	4.5
Stomach	0.1	1.1	0.2	0.2
Small intestine	0.0	0.1	0.0	0.0
Colon ca. SW480	0.6	1.0	7.0	1.4
Colon ca.* SW620 (SW480 met)	0.0	0.0	0.0	0.0
Colon ca. HT29	0.0	0.0	0.0	0.0
Colon ca. HCT-116	0.0	0.0	0.0	0.0
Colon ca. CaCo-2	0.0	0.1	0.0	0.0
Colon ca. HCT-15	0.0	0.1	0.0	0.0
Colon ca. HCC-2998	0.0	0.0	0.0	0.0
Gastric ca. (liver met) NCI-N87	0.0	0.4	0.0	0.0
Bladder	0.3	1.0	0.0	0.1
Trachea	0.0	0.4	0.0	0.0
Kidney	0.2	0.9	1.2	1.0
Kidney (fetal)	1.3	3.9	3.2	2.7
Renal ca. 786-0	10.6	11.7	7.9	12.2
Renal ca. A498	0.0	0.1	0.0	0.0
Renal ca. RXF 393	17.9	14.0	16.6	15.2
Renal ca. ACHN	0.0	0.0	0.0	0.0
Renal ca. UO-31	0.0	0.2	0.0	0.2
Renal ca. TK-10	0.0	0.0	0.0	0.0
Liver	0.0	3.2	0.9	1.9

Liver (fetal)	0.0	0.1	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0	0.0	0.0
Lung	0.0	0.2	0.0	0.0
Lung (fetal)	0.0	0.5	0.0	0.0
Lung ca. (small cell) LX-1	0.0	0.0	0.0	0.0
Lung ca. (small cell) NCI-H69	2.0	3.3	5.4	2.9
Lung ca. (s.cell var.) SHP-77	0.0	0.0	0.0	0.0
Lung ca. (large cell)NCI-H460	0.0	0.0	0.7	0.3
Lung ca. (non-sm. cell) A549	0.0	0.0	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	0.0	0.0	0.0	0.0
Lung ca. (non-s.cell) HOP-62	5.2	4.7	11.3	6.7
Lung ca. (non-s.cl) NCI-H522	1.7	3.1	2.8	4.8
Lung ca. (squam.) SW 900	0.0	0.0	0.0	0.0
Lung ca. (squam.) NCI-H596	1.3	2.5	4.2	3.0
Mammary gland	11.9	9.1	7.0	10.6
Breast ca.* (pl.ef) MCF-7	0.0	0.0	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	0.0	0.1	0.0	0.0
Breast ca.* (pl. ef) T47D	11.8	7.5	31.6	31.4
Breast ca. BT-549	0.0	0.0	13.8	28.3
Breast ca. MDA-N	0.0	0.1	0.0	0.0
Ovary	0.1	0.6	0.1	0.2
Ovarian ca. OVCAR-3	0.0	0.0	0.0	0.0
Ovarian ca. OVCAR-4	0.0	0.1	4.4	0.0
Ovarian ca. OVCAR-5	73.2	38.2	57.0	36.1
Ovarian ca. OVCAR-8	0.0	0.7	3.4	0.2
Ovarian ca. IGROV-1	0.0	0.0	2.6	0.0
Ovarian ca. (ascites) SK-OV-3	0.0	0.0	0.0	0.0
Uterus	0.4	1.2	1.7	2.9
Placenta	0.0	0.1	0.0	0.0
Prostate	0.9	1.9	1.9	1.7
Prostate ca.* (bone met) PC-3	0.0	0.0	0.0	0.0
Testis	25.7	22.2	0.7	26.4
Melanoma Hs688(A).T	23.8	21.6	20.0	28.9
Melanoma* (met) Hs688(B).T	4.6	6.5	9.6	9.2
Melanoma UACC-62	0.0	0.0	0.0	0.0
Melanoma M14	0.0	0.1	0.0	0.0
Melanoma LOX IMVI	3.7	3.4	0.2	2.7
Melanoma* (met) SK-MEL-5	0.0	0.0	0.0	0.0

Melanoma SK-MEL-28	0.0	0.0	17.4	0.0

Table AH. Panel 1.1

Column A - Rel. Exp.(%) Ag047, Run 109663520			
Tissue Name	**A**	**Tissue Name**	**A**
Adrenal gland	0.5	Renal ca. UO-31	0.1
Bladder	1.2	Renal ca. RXF 393	8.7
Brain (amygdala)	2.8	Liver	1.3
Brain (cerebellum)	4.1	Liver (fetal)	0.0
Brain (hippocampus)	9.6	Liver ca. (hepatoblast) HepG2	0.0
Brain (substantia nigra)	15.0	Lung	0.1
Brain (thalamus)	4.7	Lung (fetal)	0.4
Cerebral Cortex	48.3	Lung ca. (non-s.cell) HOP-62	26.6
Brain (fetal)	21.2	Lung ca. (large cell)NCI-H460	1.2
Brain (whole)	9.0	Lung ca. (non-s.cell) NCI-H23	0.0
glio/astro U-118-MG	0.7	Lung ca. (non-s.cl) NCI-H522	5.8
astrocytoma SF-539	38.4	Lung ca. (non-sm. cell) A549	0.0
astrocytoma SNB-75	16.4	Lung ca. (s.cell var.) SHP-77	0.0
astrocytoma SW1783	19.1	Lung ca. (small cell) LX-1	0.0
glioma U251	55.5	Lung ca. (small cell) NCI-H69	5.3
glioma SF-295	8.7	Lung ca. (squam.) SW 900	0.0
glioma SNB-19	**100.0**	Lung ca. (squam.) NCI-H596	5.0
glio/astro U87-MG	3.3	Lymph node	0.3
neuro*; met SK-N-AS	0.0	Spleen	0.0
Mammary gland	2.0	Thymus	0.6
Breast ca. BT-549	6.4	Ovary	0.6
Breast ca. MDA-N	0.0	Ovarian ca. IGROV-1	0.0
Breast ca.* (pl. ef) T47D	5.7	Ovarian ca. OVCAR-3	0.0
Breast ca.* (pl.ef) MCF-7	0.0	Ovarian ca. OVCAR-4	0.1
Breast ca.* (pl.ef) MDA-MB-231	0.0	Ovarian ca. OVCAR-5	49.0
Small intestine	0.0	Ovarian ca. OVCAR-8	0.5
Colorectal	0.1	Ovarian ca. (ascites) SK-OV-3	0.1
Colon ca. HT29	0.0	Pancreas	0.6
Colon ca. CaCo-2	0.0	Pancreatic ca. CAPAN 2	0.0
Colon ca. HCT-15	0.1	Pituitary gland	0.5
Colon ca. HCT-116	0.0	Placenta	0.0
Colon ca. HCC-2998	0.0	Prostate	0.8

Colon ca. SW480	1.2	Prostate ca.* (bone met) PC-3	0.1
Colon ca.* SW620 (SW480 met)	0.0	Salivary gland	0.2
Stomach	0.2	Trachea	0.5
Gastric ca. (liver met) NCI-N87	0.1	Spinal cord	1.4
Heart	80.7	Testis	0.5
Skeletal muscle (Fetal)	1.6	Thyroid	0.3
Skeletal muscle	0.4	Uterus	0.0
Endothelial cells	0.0	Melanoma M14	0.0
Heart (Fetal)	14.2	Melanoma LOX IMVI	0.7
Kidney	2.4	Melanoma UACC-62	0.0
Kidney (fetal)	1.5	Melanoma SK-MEL-28	0.0
Renal ca. 786-0	3.6	Melanoma* (met) SK-MEL-5	0.0
Renal ca. A498	0.0	Melanoma Hs688(A).T	16.5
Renal ca. ACHN	0.0	Melanoma* (met) Hs688(B).T	5.6
Renal ca. TK-10	0.0		

Table AI. Panel 2.2

Column A - Rel. Exp.(%) Ag2975, Run 173763053			
Tissue Name	**A**	**Tissue Name**	**A**
Normal Colon	2.5	Kidney Margin (OD04348)	29.7
Colon cancer (OD06064)	4.7	Kidney malignant cancer (OD06204B)	0.0
Colon Margin (OD06064)	4.3	Kidney normal adjacent tissue (OD06204E)	0.0
Colon cancer (OD06159)	2.2	Kidney Cancer (OD04450-01)	0.0
Colon Margin (OD06159)	7.3	Kidney Margin (OD04450-03)	13.6
Colon cancer (OD06297-04)	0.0	Kidney Cancer 8120613	2.3
Colon Margin (OD06297-05)	0.0	Kidney Margin 8120614	11.5
CC Gr.2 ascend colon (ODO3921)	0.0	Kidney Cancer 9010320	23.8
CC Margin (ODO3921)	0.0	Kidney Margin 9010321	20.0
Colon cancer metastasis (OD06104)	0.0	Kidney Cancer 8120607	2.0
Lung Margin (OD06104)	0.0	Kidney Margin 8120608	0.0
Colon mets to lung (OD04451-01)	0.0	Normal Uterus	4.4
Lung Margin (OD04451-02)	2.0	Uterine Cancer 064011	0.0
Normal Prostate	7.5	Normal Thyroid	4.4
Prostate Cancer (OD04410)	2.3	Thyroid Cancer	0.0
Prostate Margin (OD04410)	4.6	Thyroid Cancer A302152	2.2
Normal Ovary	2.2	Thyroid Margin A302153	3.6

Ovarian cancer (OD06283-03)	0.0	Normal Breast	55.1
Ovarian Margin (OD06283-07)	0.0	Breast Cancer	3.9
Ovarian Cancer	13.4	Breast Cancer	68.3
Ovarian cancer (OD06145)	18.8	Breast Cancer (OD04590-01)	5.3
Ovarian Margin (OD06145)	8.1	Breast Cancer Mets (OD04590-03)	0.0
Ovarian cancer (OD06455-03)	5.0	Breast Cancer Metastasis	13.1
Ovarian Margin (OD06455-07)	2.3	Breast Cancer	12.5
Normal Lung	2.3	Breast Cancer 9100266	34.6
Invasive poor diff. lung adeno (ODO4945-01	0.0	Breast Margin 9100265	17.0
Lung Margin (ODO4945-03)	0.0	Breast Cancer A209073	16.7
Lung Malignant Cancer (OD03126)	1.4	Breast Margin A209073	71.7
Lung Margin (OD03126)	0.0	Breast cancer (OD06083)	20.7
Lung Cancer (OD05014A)	0.0	Breast cancer node metastasis (OD06083)	6.3
Lung Margin (OD05014B)	2.1	Normal Liver	0.0
Lung cancer (OD06081)	**100.0**	Liver Cancer 1026	8.1
Lung Margin (OD06081)	4.1	Liver Cancer 1025	29.5
Lung Cancer (OD04237-01)	0.0	Liver Cancer 6004-T	8.4
Lung Margin (OD04237-02)	0.0	Liver Tissue 6004-N	4.7
Ocular Mel Met to Liver (ODO4310)	6.7	Liver Cancer 6005-T	23.0
Liver Margin (ODO4310)	2.8	Liver Tissue 6005-N	32.8
Melanoma Metastasis	2.2	Liver Cancer	12.8
Lung Margin (OD04321)	3.7	Normal Bladder	0.0
Normal Kidney	9.0	Bladder Cancer	6.3
Kidney Ca, Nuclear grade 2 (OD04338)	19.5	Bladder Cancer	16.7
Kidney Margin (OD04338)	3.1	Normal Stomach	15.3
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Gastric Cancer 9060397	0.0
Kidney Margin (OD04339)	9.3	Stomach Margin 9060396	3.8
Kidney Ca, Clear cell type (OD04340)	0.0	Gastric Cancer 9060395	6.5
Kidney Margin (OD04340)	4.6	Stomach Margin 9060394	2.3
Kidney Ca, Nuclear grade 3 (OD04348)	77.4	Gastric Cancer 064005	0.0

Table AJ. Panel 2D

Column A - Rel. Exp.(%) Ag047. Run 144771648

Column B - Rel. Exp.(%) Ag047, Run 152940364 C lumn C - Rel. Exp.(%) Ag2679, Run 158633803 Column D - Rel. Exp.(%) Ag2728, Run 158561830				
Tissue Name	**A**	**B**	**C**	**D**
Normal Colon	5.5	8.9	7.4	10.4
CC Well to Mod Diff (ODO3866)	1.4	0.2	0.2	1.3
CC Margin (ODO3866)	0.4	0.5	0.4	0.1
CC Gr.2 rectosigmoid (ODO3868)	0.4	0.1	1.7	0.2
CC Margin (ODO3868)	2.5	0.9	0.7	0.7
CC Mod Diff (ODO3920)	0.0	0.0	0.0	0.2
CC Margin (ODO3920)	0.7	0.9	1.1	0.3
CC Gr.2 ascend colon (ODO3921)	0.0	0.7	0.6	0.2
CC Margin (ODO3921)	0.2	0.2	0.6	0.8
CC from Partial Hepatectomy (ODO4309) Mets	0.6	0.4	1.1	1.0
Liver Margin (ODO4309)	11.1	13.6	30.4	21.2
Colon mets to lung (OD04451-01)	0.0	0.4	0.1	0.2
Lung Margin (OD04451-02)	1.3	1.0	0.5	0.7
Normal Prostate 6546-1	19.1	0.7	1.0	2.3
Prostate Cancer (OD04410)	5.8	2.9	4.7	3.4
Prostate Margin (OD04410)	4.7	5.7	7.2	4.9
Prostate Cancer (OD04720-01)	2.9	3.3	3.4	3.3
Prostate Margin (OD04720-02)	12.5	10.7	8.3	15.2
Normal Lung	1.5	2.7	6.8	5.4
Lung Met to Muscle (ODO4286)	0.3	0.1	0.0	0.5
Muscle Margin (ODO4286)	0.3	0.2	0.3	0.6
Lung Malignant Cancer (OD03126)	0.4	0.2	0.8	1.5
Lung Margin (OD03126)	0.4	1.0	1.2	0.5
Lung Cancer (OD04404)	86.5	**100.0**	**100.0**	**100.0**
Lung Margin (OD04404)	18.3	3.3	2.2	3.0
Lung Cancer (OD04565)	**100.0**	52.1	62.0	77.9
Lung Margin (OD04565)	0.2	0.1	0.0	0.6
Lung Cancer (OD04237-01)	6.3	1.5	3.2	3.3
Lung Margin (OD04237-02)	1.4	0.5	0.5	0.6
Ocular Mel Met to Liver (ODO4310)	0.4	0.3	0.5	0.7
Liver Margin (ODO4310)	2.3	1.8	3.5	2.8
Melanoma Metastasis	0.0	0.3	0.4	1.6
Lung Margin (OD04321)	2.1	2.6	2.2	3.0
Normal Kidney	6.7	4.9	8.4	7.0

Kidney Ca, Nuclear grade 2 (OD04338)	0.0	0.0	0.3	1.4
Kidney Margin (OD04338)	3.5	1.5	4.1	3.5
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.1	0.3	0.7
Kidney Margin (OD04339)	18.4	10.3	8.4	15.5
Kidney Ca, Clear cell type (OD04340)	0.0	0.8	0.7	1.1
Kidney Margin (OD04340)	6.5	4.4	4.5	6.5
Kidney Ca, Nuclear grade 3 (OD04348)	90.8	36.1	54.3	50.3
Kidney Margin (OD04348)	4.6	3.2	3.8	4.0
Kidney Cancer (OD04622-01)	2.7	1.8	4.1	3.5
Kidney Margin (OD04622-03)	2.0	0.2	0.3	1.1
Kidney Cancer (OD04450-01)	0.0	0.0	0.0	0.0
Kidney Margin (OD04450-03)	3.1	1.4	6.9	5.4
Kidney Cancer 8120607	1.5	0.3	0.5	1.8
Kidney Margin 8120608	3.4	0.8	1.3	2.2
Kidney Cancer 8120613	0.4	0.8	4.3	2.0
Kidney Margin 8120614	2.8	1.2	4.9	4.0
Kidney Cancer 9010320	76.8	39.0	36.3	52.9
Kidney Margin 9010321	10.4	5.1	4.9	4.3
Normal Uterus	0.0	0.2	0.3	0.0
Uterine Cancer 064011	0.9	0.0	0.3	1.9
Normal Thyroid	0.3	0.0	0.9	2.2
Thyroid Cancer	0.0	0.0	0.0	0.0
Thyroid Cancer A302152	1.4	0.1	0.4	0.0
Thyroid Margin A302153	1.7	0.9	6.4	4.1
Normal Breast	20.3	5.7	13.0	20.9
Breast Cancer	0.3	0.1	0.5	0.3
Breast Cancer (OD04590-01)	2.4	2.2	1.1	1.9
Breast Cancer Mets (OD04590-03)	0.7	0.1	0.3	0.0
Breast Cancer Metastasis	6.9	3.3	7.7	9.3
Breast Cancer	5.6	8.3	4.2	4.9
Breast Cancer	47.0	19.3	30.1	23.5
Breast Cancer 9100266	14.9	9.3	15.7	21.8
Breast Margin 9100265	4.6	1.5	4.4	8.4
Breast Cancer A209073	48.3	12.9	28.3	40.9
Breast Margin A209073	38.7	16.6	27.5	29.7
Normal Liver	0.2	0.1	0.0	0.2
Liver Cancer	11.8	5.6	4.6	4.5
Liver Cancer 1025	4.5	1.6	4.2	2.8

Liver Cancer 1026	6.2	6.7	9.0	6.4
Liver Cancer 6004-T	15.6	3.3	3.5	2.8
Liver Tissue 6004-N	0.1	0.2	0.2	0.4
Liver Cancer 6005-T	14.6	8.0	8.7	7.9
Liver Tissue 6005-N	6.4	7.0	5.9	3.2
Normal Bladder	1.3	0.9	1.6	0.9
Bladder Cancer	0.4	0.2	0.3	0.3
Bladder Cancer	7.5	5.3	12.1	19.8
Bladder Cancer (OD04718-01)	27.7	23.0	35.6	41.5
Bladder Normal Adjacent (OD04718-03)	0.4	0.3	0.6	0.7
Normal Ovary	1.4	0.4	1.1	0.3
Ovarian Cancer	1.3	0.5	1.3	1.3
Ovarian Cancer (OD04768-07)	0.4	0.2	0.0	0.0
Ovary Margin (OD04768-08)	1.0	0.8	0.6	1.6
Normal Stomach	4.3	2.0	0.3	3.1
Gastric Cancer 9060358	2.4	0.7	0.7	1.2
Stomach Margin 9060359	0.0	0.0	0.7	4.3
Gastric Cancer 9060395	0.9	2.0	1.9	2.4
Stomach Margin 9060394	0.2	0.3	1.1	0.2
Gastric Cancer 9060397	0.4	0.5	1.6	0.7
Stomach Margin 9060396	0.0	0.1	0.2	0.2
Gastric Cancer 064005	0.7	0.7	0.9	1.5

Table AK. Panel 3D

Column A - Rel. Exp.(%) Ag047, Run 158634002			
Tissue Name	**A**	**Tissue Name**	**A**
94905 Daoy Medulloblastoma/Cerebellum	0.9	94954 Ca Ski Cervical epidermoid carcinoma (metastasis	0.3
94906 TE671 Medulloblastom/Cerebellum	0.0	94955 ES-2 Ovarian clear cell carcinoma	6.7
94907 D283 Med Medulloblastoma/Cerebellum	0.0	94957 Ramos Stimulated with PMA/ionomycin 6h	0.0
94908 PFSK-1 Primitive Neuroectodermal/Cerebellum	0.0	94958 Ramos Stimulated with PMA/ionomycin 14h	0.0
94909 XF-498 CNS	0.0	94962 MEG-01 Chronic myelogenous leukemia (megokaryoblast)	0.0
94910 SNB-78 CNS/glioma	**100.0**	94963 Raji Burkitt's lymphoma	0.0
94911 SF-268 CNS/glioblastoma	79.0	94964 Daudi Burkitt's lymphoma	0.0

94912 T98G Glioblastoma	1.4	94965 U266 B-cell plasmacytoma/myeloma	0.0
96776 SK-N-SH Neuroblastoma (metastasis)	2.0	94968 CA46 Burkitt's lymphoma	0.0
94913 SF-295 CNS/glioblastoma	0.1	94970 RL non-Hodgkin's B-cell lymphoma	0.0
94914 Cerebellum	4.5	94972 JM1 pre-B-cell lymphoma/leukemia	0.0
96777 Cerebellum	2.9	94973 Jurkat T cell leukemia	0.0
94916 NCI-H292 Mucoepidermoid lung carcinoma	3.8	94974 TF-1 Erythroleukemia	0.0
94917 DMS-114 Small cell lung cancer	0.0	94975 HUT 78 T-cell lymphoma	0.0
94918 DMS-79 Small cell lung cancer/neuroendocrine	0.1	94977 U937 Histiocytic lymphoma	0.0
94919 NCI-H146 Small cell lung cancer/neuroendocrine	0.0	94980 KU-812 Myelogenous leukemia	0.0
94920 NCI-H526 Small cell lung cancer/neuroendocrine	1.0	769-P- Clear cell renal carcinoma	0.1
94921 NCI-N417 Small cell lung cancer/neuroendocrine	0.0	94983 Caki-2 Clear cell renal carcinoma	0.1
94923 NCI-H82 Small cell lung cancer/neuroendocrine	7.6	94984 SW 839 Clear cell renal carcinoma	0.0
94924 NCI-H157 Squamous cell lung cancer (metastasis)	0.8	94986 G401 Wilms' tumor	6.0
94925 NCI-H1155 Large cell lung cancer/neuroendocrine	0.0	94987 Hs766T Pancreatic carcinoma (LN metastasis)	0.0
94926 NCI-H1299 Large cell lung cancer/neuroendocrine	0.2	94988 CAPAN-1 Pancreatic adenocarcinoma (liver metastasis)	0.0
94927 NCI-H727 Lung carcinoid	0.0	94989 SU86.86 Pancreatic carcinoma (liver metastasis)	0.1
94928 NCI-UMC-11 Lung carcinoid	0.0	94990 BxPC-3 Pancreatic adenocarcinoma	0.6
94929 LX-1 Small cell lung cancer	0.0	94991 HPAC Pancreatic adenocarcinoma	0.0
94930 Colo-205 Colon cancer	0.0	94992 MIA PaCa-2 Pancreatic carcinoma	0.0
94931 KM12 Colon cancer	0.0	94993 CFPAC-1 Pancreatic ductal adenocarcinoma	0.1
94932 KM20L2 Colon cancer	0.0	94994 PANC-1 Pancreatic epithelioid ductal carcinoma	0.0
94933 NCI-H716 Colon cancer	0.0	94996 T24 Bladder carcinma	0.0

		(transitional cell	
94935 SW-48 Colon adenocarcinoma	0.0	5637- Bladder carcinoma	3.8
94936 SW1116 Colon adenocarcinoma	0.0	94998 HT-1197 Bladder carcinoma	0.0
94937 LS 174T Colon adenocarcinoma	0.0	94999 UM-UC-3 Bladder carcinma (transitional cell)	10.3
94938 SW-948 Colon adenocarcinoma	0.0	95000 A204 Rhabdomyosarcoma	0.0
94939 SW-480 Colon adenocarcinoma	0.0	95001 HT-1080 Fibrosarcoma	59.9
94940 NCI-SNU-5 Gastric carcinoma	0.7	95002 MG-63 Osteosarcoma (bone)	0.5
KATO III- Gastric carcinoma	0.0	95003 SK-LMS-1 Leiomyosarcoma (vulva)	4.2
94943 NCI-SNU-16 Gastric carcinoma	0.2	95004 SJRH30 Rhabdomyosarcoma (met to bone marrow)	0.3
94944 NCI-SNU-1 Gastric carcinoma	0.0	95005 A431 Epidermoid carcinoma	0.6
94946 RF-1 Gastric adenocarcinoma	0.3	95007 WM266-4 Melanoma	0.0
94947 RF-48 Gastric adenocarcinoma	0.1	DU 145- Prostate carcinoma (brain metastasis)	0.0
96778 MKN-45 Gastric carcinoma	0.0	95012 MDA-MB-468 Breast adenocarcinoma	0.0
94949 NCI-N87 Gastric carcinoma	0.0	SCC-4- Squamous cell carcinoma of tongue	0.2
94951 OVCAR-5 Ovarian carcinoma	0.0	SCC-9- Squamous cell carcinoma of tongue	1.1
94952 RL95-2 Uterine carcinoma	4.0	SCC-15- Squamous cell carcinoma of tongue	0.3
94953 HelaS3 Cervical adenocarcinoma	0.0	95017 CAL 27 Squamous cell carcinoma of tongue	7.0

RESULTS

Gene expression was consistently detected in a certain cancer specimens throughout these studies. Specifically, the gene was expressed in brain cancer (astrocytoma and glioma), renal, ovarian, melanoma cell lines in Panels 1 and 1.1. Panel 3D shows expression in additional glioma and a fibrosarcoma cell lines. Clinical specimens of ovarian, lung, kidney, liver, breast and bladder cancers express this gene as seen in Panels 2.2 and 2D. Therefore expression of this gene can be used to differentiate these pathological cells and tissues from normal tissue and as a diagnostic marker for the presence of these cancers.

Panel 1: Significant gene expression was detected in astrocytoma cell lines SW1783, SF-539, SNB-75 and glioma cell lines SNB-19 and U251. While significant expression was detected in a normal whole brain sample in 3 out of 4 experiments, it was not seen in

samples from specific areas of the brain such as amygdala, cerebellum, hippocampus, substantia nigra, thalamus and hypothalamus nor was it seen in fetal brain tissue. Normal and fetal kidney showed no expression of this gene while renal carcinoma cell lines 786-0 and RXF393 had positive expression. Similarly normal ovarian tissue did not express this gene however expression was detected in OVCAR-5 ovarian cancer cell line. The gene was also expressed in melanoma cell line Hs688(A).T. The gene was not detected in other types of cancer cell lines for example colon, liver, small cell lung, and prostate.

Panel 1.1: Confirms gene expression in: astrocytoma cell lines SF-539, moderately expression in SNB-75, SW1783; glioma cell lines U251 and SNB-19; OVCAR-5; and melanoma Hs688(A).T. This experiment also detected expression in non small cell lung cell line HOP-62.

Panel 2.2: When clinical cancer specimens were studied compared to margin or normal tissue, differential expression was detected in ovarian, lung (type unspecified), kidney (Nuclear grade 2 and 3), liver and bladder carcinoma. Expression was also detected in some breast cancer specimens but also in margin specimens and normal breast. Expression was not detected in the one specimen of prostate carcinoma.

Panel 2D investigated expression in additional pathology specimens and results showed differential expression in lung, kidney (particularly Nuclear grade 3), two breast, one liver, and one bladder carcinomas. Expression was not detected in the limited samples of colon, prostate, uterine, thyroid, ovarian, and gastric carcinoma specimens.

Panel 3D is a collection of additional carcinoma cell lines and results showed gene expression in glioma cell lines SNB-78 and SF-268 and fibrosarcoma HT-1080.